Identification and mapping of two powdery mildew resistance genes in Triticum boeoticum L

Chhuneja, P ; Kumar, K ; Stirnweis, D ; Hurni, S ; Keller, B ; Dhaliwal, H S ; Singh, K

Abstract: Powdery mildew (PM) caused by Blumeria graminis f. sp. tritici (Bgt), is one of the important foliar diseases of wheat that can cause serious yield losses. Breeding for cultivars with diverse resources of resistance is the most promising approach for combating this disease. The diploid A genome progenitor species of wheat are an important resource for new variability for disease resistance genes. An accession of Triticum boeoticum (A(b)A(b)) showed resistance against a number of Bgt isolates, when tested using detached leaf segments. Inheritance studies in a recombinant inbred line population (RIL), developed from crosses of PM resistant T. boeoticum acc. pau5088 with a PM susceptible T. monococcum acc. pau14087, indicated the presence of two powdery mildew resistance genes in T. boeoticum acc. pau5088. Analysis of powdery mildew infection and molecular marker data of the RIL population revealed that both powdery mildew resistance genes are located on the long arm of chromosome 7A. Mapping was conducted using an integrated linkage map of 7A consisting of SSR, RFLP, STS, and DArT markers. These powdery mildew resistance genes are tentatively designated as PmTb7A.1 and PmTb7A.2. The PmTb7A.2 is closely linked to STS markers MAG2185 and MAG1759 derived from RFLP probes which are linked to powdery mildew resistance gene Pm1. This indicated that PmTb7A.2 might be allelic to Pm1. The PmTb7A.1, flanked by a DArT marker wPt4553 and an SSR marker Xcfa2019 in a 4.3 cM interval, maps proximal to PmT7A.2. PmTb7A.1 is putatively a new powdery mildew resistance gene. The powdery mildew resistance genes from T. boeoticum are currently being transferred to cultivated wheat background through marker-assisted backcrossing, using T. durum as bridging species.

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Identification and mapping of two powdery mildew resistance genes in *Triticum boeoticum* L.

Parveen Chhuneja[1], Krishan Kumar[1], Daniel Stirnweis[2], Severine Hurni[2], Beat Keller[2], Harcharan S Dhaliwal[1$] and Kuldeep Singh[1§]

[1]School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana 141 004, India

[2]Institute of Plant Biology, University of Zurich, Switzerland

[$] Present address: Akal School of Biotechnology, Eternal University, Baru Sahib, Himachal Pradesh-173101, India

[§]Corresponding author email: kuldeep35@pau.edu

Telephone: 91-161-2401960 Ext. 270

Fax: 91-161-2401444

Abstract

Powdery mildew (PM) caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is one of the important foliar diseases of wheat that can cause serious yield losses. Breeding for cultivars with diverse resources of resistance is the most promising approach for combating this disease. The diploid A-genome progenitor species of wheat are an important resource for new variability for disease resistance genes. An accession of *Triticum boeoticum* (A^bA^b) showed resistance against a number of *Bgt* isolates, when tested using detached leaf segments. Inheritance studies in a recombinant inbred line population (RIL), developed from crosses of PM resistant *T. boeoticum* acc. pau5088 with a PM susceptible *T. monococcum* acc. pau14087, indicated the presence of two powdery mildew resistance genes in *T. boeoticum* acc. pau5088. Analysis of powdery mildew infection and molecular marker data of the RIL population revealed that both powdery mildew resistance genes are located on the long arm of chromosome 7A. Mapping was conducted using an integrated linkage map of 7A consisting of SSR, RFLP, STS, and DArT markers. These powdery mildew resistance genes are tentatively designated as *PmTb7A.1* and *PmTb7A.2*. The *PmTb7A.2* is closely linked to STS markers MAG2185 and MAG1759 derived from an RFLP probes which are linked to powdery mildew resistance gene *Pm1*. This indicated

that *PmTb7A.2* might be allelic to *Pm1*. The *PmTb7A.1,* flanked by a DArT marker *wPt4553* and an SSR marker *Xcfa2019* in a 4.3cM interval, maps proximal to *PmT7A.2*. *PmTb7A.1* is putatively a new powdery mildew resistance gene. The powdery mildew resistance genes from *T. boeoticum* are currently being transferred to cultivated wheat background through marker-assisted backcrossing, using *T. durum* as bridging species.

Keywords: Disease resistance, Powdery mildew, *Triticum boeoticum*, molecular mapping

Introduction

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a foliar disease of wheat occuring worldwide. It competes for nutrients and reduces the photosynthetic capacity of the leaves. The cultivation of high-yielding varieties and improved irrigation and fertilization conditions has further intensified the threat of powdery mildew. Severe epidemics of this disease often occur in areas with cool and humid climates, causing significant yield losses (Bennett 1984). Breeding and deployment of resistant cultivars is the economical and environmentally friendly method to avoid fungicide applications and reduction in the yield. The discovery and utilization of new powdery mildew resistance genes has been the long-term objective for wheat geneticists and breeders worldwide. To date, more than 40 powdery mildew resistance genes designated as *Pm1–Pm43* have been identified (McIntosh et al. 2008) and most of these genes show race specific resistance. Among these, the *Pm3* resistance alleles have been well characterized and cloned (Yahiaoui et al. 2004; 2006). Four powdery mildew resistance genes (*Pm1, Pm3, Pm4,* and *Pm5*) have more than one allele conferring resistance. A number of known powdery mildew resistance genes are no longer effective due to the emergence of new virulent races. In addition, some of the highly effective genes are either associated with linkage drag or are only available in unadapted backgrounds, thus limiting their usefulness in the breeding programmes (Duan et al. 1998; Qiu and Zhang 2004). Most of the Indian durum and bread wheat cultivars are highly susceptible to powdery mildew.

The diploid "A" genome progenitor gene pool of wheat, comprising the three closely related species *Triticum monococcum* ssp *monococcum* (*T. monococcum*), *T. monococcum* ssp *aegilopoides* (*Triticum boeoticum*) and *Triticum urartu*, harbours useful variability for many economically important genes, including resistance to diseases (Feldman and Sears 1981; Dhaliwal et al. 1993; Hussien et al. 1997; Singh et al. 2007a). Many of the powdery mildew

resistance genes such as *Pm1a, Pm1b* and *Pm25* have been introgressed from diploid A genome progenitor species. Likewise, genes such as *Pm4a, Pm4b, Pm5a, Pm6, Pm16, Pm26, Pm27, Pm30, Pm31* and *Pm33* have been transferred from tetraploid wheat (McIntosh et al. 2008). Srnic et al. (2005) reported the identification of two dominant resistance genes from *T. monococcum* L. and *Triticum timopheevii* L. Yao et al. (2007) identified two new alleles of *Pm1* from two different accessions of *T. monococcum*. Xu et al. (2008) mapped and introgressed a recessive powdery mildew resistance gene from *T. monococcum* on chromosome 5AL. Since it is relatively easy to transfer genes from ancestor species of wheat, these have been extensively studied for the identification of new alleles and genes useful for wheat improvement. Currently, molecular markers for more than 29 powdery mildew resistance genes have been reported (McIntosh et al. 2008; 2010). Some of these markers have been successfully used in map-based cloning (Yahiaoui et al. 2004), marker-assisted selection and pyramiding of the resistance genes (Liu et al. 2000).

In this study the *T. boeoticum* acc. pau5088 was crossed with *T. monococcum* accession pau14087 to generate a RIL population, which showed segregation for stripe rust, Karnal bunt and cereal cyst nematode resistance (Singh et al. 2007a; Chhuneja et al. 2008; Singh et al. 2010). This population was used for generating a linkage map of the diploid A genome of wheat (Singh et al. 2007b) and the mapping of these disease resistance genes. *T. boeoticum* acc. pau5088 was identified to be resistant to powdery mildew also whereas *T. monococcum* was susceptible. Here, we report on the identification, genetic analysis and mapping of two powdery mildew resistance genes in *T. boeoticum* acc. pau5088.

Material and Methods

Plant Material

The plant material used for studying inheritance and mapping of the powdery mildew resistance genes consisted of a set of 148 recombinant inbred lines (RILs) derived from the cross *T. boeoticum* acc. pau5088/*T. monococcum* acc. pau14087 (hereafter referred as *Tb*5088 and *Tm*14087, respectively for brevity) through single seed descent. Detailed information on these accessions and a molecular linkage map generated using this population was described by Singh et al. (2007b) and is also available at GrainGenes (http://wheat.pw.usda.gov/ggpages

/map_summary.html). Of the 148 RILs, 92 were used for generating the linkage map, whereas all the RILs were screened against three powdery mildew pathotypes.

Screening against powdery mildew

Powdery mildew screening was conducted following a modified protocol as described in Limpert (1987). Briefly, detached leaves from ten day old plants were placed on phytagar media (0.5% phytagar; 30ppm Benzimidazole), inoculated with *Bgt* pathotypes and incubated at 20°C, 16h light/8h darkness. The data was recorded ten days after inoculations. Infection was scored as the percentage of diseased leaf surface. The RILs with powdery mildew score of 0-5% were categorized as resistant (R) and RILs with >5% PM score were classified as susceptible (S). The wheat powdery mildew isolates were used from the former mildew collections of Agroscope Reckenholz-Tänikon ART, Switzerland (*Bgt* 96224, *Bgt* 97011, *Bgt* 98229, *Bgt* 98275; http://www.art.admin.ch) and INRA Rennes, France (*Bgt* 95.9Asosan; http://www.rennes.inra.fr), from USDA-ARS North Carolina State University, Raleigh (*Bgt* Ken2-5, *Bgt* J2-1, *Bgt* C3-1, *Bgt* 85063; http://www.ars.usda.gov/saa/psru) and from an isolate collection by Brunner et al. (2010) (Bgt 07201, Bgt 07230, Bgt 07928, Bgt 07302 and Bgt 07286). Isolates were propagated on the hexaploid wheat variety "Kanzler". The parental accessions and the RIL population were screened against different *Bgt* isolates using the detached leaf method. *Tm*14087, *Tb*5088 along with susceptible controls *T. aestivum* cultivars WL711, and PBW343 and two RILs were screened against 14 different isolates. Bread wheat cultivars WL711 and PBW343, as well as RIL101 and RIL130 were chosen because we have developed a series of introgression lines by crossing WL711 and PBW343 with RIL101 and RIL130 using *T. durum* as bridging species (Singh et al. 2007a; Chhuneja et al. 2008). Three of the isolates *Bgt* 07201, *Bgt* 07302 and *Bgt* 97011 showing differential reaction on the parental lines were selected to screen the RILs for studying the inheritance of powdery mildew resistance in the *Tb*5088/*Tm*14087 population.

Mapping of powdery mildew resistance

The phenotypic score was used for mapping powdery mildew resistance using only a set of 92 RILs of the *Tb*5088/*Tm*14087 RIL population. The linkage map consisting of 188 markers was used for mapping PM resistance genes with composite interval mapping (CIM) using MapManager QTXb20 (Manly et al. 2001). Since the population segregated for two PM

resistance genes, Mapmaker could not be used for mapping of the powdery mildew resistance in this population. In CIM analysis, phenotypic data of the RILs for three *Bgt* isolates were entered along with the genotypic data of the RILs. The "marker regression" function ($P = 0.01$) was used to detect possible single marker locus associated with the powdery mildew resistance QTL. The locus with the highest likelihood ratio statistic (LRS) for each set of data was added to the background and composite interval mapping used for localization and estimation of effects of each QTL after correcting the effect of background loci. Significant ($P < 0.05$) and highly significant ($P < 0.01$) threshold levels were determined by the permutation test function of MapManager, which is based on the statistical methods developed by Churchill and Doerge (1994). Phenotypic variance explained by each QTL (R^2) was estimated for each data set as difference between the total variance and the residual variance expressed as the percent of the total variance. In addition to MapManager QTXb20, IciMapping 3.1 (Wang et al. 2011) was also used to confirm location and the contribution of the QTL.

Marker enrichment on chromosome 7AL of *Tb*5088/*Tm*14087 RIL population

In order to saturate the regions carrying the powdery mildew resistance genes, primers designed from RFLP probes, MAG1714, MAG2166, MAG2185, MAG1757 and MAG1759, reported to be linked with powdery mildew resistance genes on chromosome 7A (Yao et al. 2007), were analysed on the RIL population. In addition, 38 primers designed from ESTs and 20 SSR primers already mapped on the distal half of 7AL (Somers et al. 2004) were analysed for parental polymorphism. Polymorphic markers were analysed on the RIL population. The PCR products were resolved on 8% native or denaturing PAGE gels. More than 650 DArT markers have also been mapped on this RIL population, details of which will be reported elsewhere. Thus, in the present study an integrated map of chromosome 7A combining all marker systems including SSRs, STS and DArT markers was generated using mapping software Joinmap® (Van Ooijen 2006) and used for fine mapping of the powdery mildew resistance genes.

Results

Inheritance of PM resistance in *T. boeoticum/T. monococcum* RIL population

The parental lines *Tm*14087 and *Tb*5088, along with RIL101, RIL130 and susceptible controls were inoculated with 14 *Bgt* isolates. *Tm*14087 was susceptible to all the isolates with disease

scores varying between 20-100% for different isolates. *Tb*5088 on the other hand was highly resistant, with no powdery mildew growth and an immune reaction to all the isolates tested (Table 1; Fig. 1). RIL101 was resistant and RIL130 was susceptible. Earlier, the powdery mildew resistance gene *Pm1* had been transferred from *T. monococcum* into cultivated wheat (Sears and Briggle 1969; McIntosh et al. 2008). To establish whether the powdery mildew resistance gene(s) identified in *Tb*5088 are different from *Pm1*, the *T. aestivum* near isogenic line Axminster/8*CC and the breeding line Weihenstephan Stamm M1N (M1N; Hsam et al. 1998) carrying the *Pm1a* and *Pm1c* alleles of *Pm1*, respectively were also tested along with the parental lines (Table 1). The Axminster/8*CC (*Pm1a*) was susceptible to all of the tested isolates except for *Bgt*J2-1, whereas M1N (*Pm1c*) was resistant to six of the eleven isolates tested. *Tb*5088, however, was resistant to all the isolates tested

The RIL population was tested with the three selected isolates *Bgt* 07201, *Bgt* 07302, and *Bgt* 97011. It segregated in a ratio of 97R:46S, 97R:45S and 97R:46S, respectively with χ^2 (3:1) values 3.91, 3.39 and 3.91 (Table 2). The RIL population thus segregated for two genes for powdery mildew resistance, both contributed by the resistant parent *Tb*5088. All the RILs had similar reaction to all three *Bgt* isolates tested indicating that both the powdery resistance genes of Tb5088 were effective against all three tested isolates.

Molecular mapping of powdery mildew resistance in *T. boeoticum* acc. pau5088

A framework linkage map, consisting of 188 SSR and RFLP loci (Singh et al. 2007b), was initially used for mapping the powdery mildew resistance gene(s) in a set of 92 RILs. Data for three individual *Bgt* isolates were used for detection and mapping of the PM resistance genes in Tb5088/Tm14087 RIL population. Since the population was segregating for two PM resistance genes it was not possible to map these genes using linkage mapping softwares such as Mapmaker. The initial mapping was conducted using a QTL mapping approach with MapManager QTXb20. Composite interval mapping detected a major locus on 7AL with LRS values of 32.6, 36.1 and 31.1 with R^2 of 28%, 31% and 27%, respectively, for isolates *Bgt* 07201, *Bgt* 07302 and *Bgt* 97011 (Table 3). The high R^2 value of 27-31% indicated that this is a major gene. However, only one chromosomal region, flanked by SSR markers *Xcfa2019* and *Xgwm344*, could be detected using the available linkage map.

Enriching the chromosome 7A map

In the linkage map of Singh et al. (2007b), chromosome 7A was split into one major and two smaller linkage groups, with the major group consisting of 20 markers and two small linkage groups with two markers each. The PM resistance mapped on one of the smaller linkage groups, which corresponded to 7AL. To fine map the powdery mildew resistance gene on 7AL and integrate the three linkage groups, an additional 43 STS markers derived from 7A specific RFLP probes and ESTs were analysed for parental polymorphism on *Tm*14087 and *Tb*5088. Out of 43 STS markers only eight were polymorphic and these were analysed on the RIL population. Likewise, out of 20 additional 7AL specific SSR markers amplified on parental lines, only six were polymorphic, whereas five did not show any amplification in *Tm*14087 and *Tb*5088. Since powdery mildew resistance gene *Pm1* was mapped on 7AL, the STS markers reported to be linked to *Pm1* gene were also included in the present investigation. A saturated linkage map of chromosome 7A was generated by integrating the markers from Singh et al. (2007b), with the 7AL specific SSRs and STS markers. A partial 7AL map consisting of 11 markers is presented in Fig. 2a. Still these markers constituted a separate linkage group which did not join the main linkage group of chromosome 7A. To further enrich chromosome 7AL, the DArT markers were also included in the linkage map. A total of 153 markers could be mapped on 7A and all these assembled in single linkage group. The partial map for 7ALwith all the markers is presented in Fig. 2b. Comparison of the two maps showed that DArT markers mapped in the intervals between SSR and STS markers. SSR and STS markers in the two maps maintained their collinearity..

Fine mapping of the PM resistance genes

The 7A linkage map consisting of SSR, STS and DArT markers was then used for mapping PM resistance gene (s) using MapManager QTXb20. CIM detected two major chromosomal regions on 7AL to be associated with PM resistance. One of the QTL mapped in the region flanked by DArT marker *wPt-4553* and SSR marker *Xcfa2019* in a 4.3 cM interval with an LRS value of 36.1, 36.2 and 33.7 and R^2 of 30%, 31% and 29%, respectively for isolates *Bgt* 07201, *Bgt* 07302 and *Bgt* 97011 (Table 4; Fig. 2). The second gene mapped in the marker interval

MAG1759-MAG2185b with LRS value of 38.2, 40.6 and 31.3 and R^2 of 32%, 34% and 27%, respectively for isolates *Bgt* 07201, *Bgt* 07302 and *Bgt* 97011 (Table 4). As per the present map, the two powdery mildew resistance genes are located at a distance of 46 cM on 7AL. Both the QTL appear to be the major genes because either of them confers complete resistance to all three tested isolates, even when present alone. Hence these are tentatively designating as *PmTb7A.1* and *PmTb7A.2*. The location and contribution of *PmTb7A.1* and *PmTb7A.2* was also confirmed using another QTL mapping software IciMapping v3.1 (data not given).

Graphical genotyping of the RILs for the chromosomal regions associated with PM resistance

Graphical genotypes for all the RILs were generated for regions associated with powdery mildew resistance. Sixty out of the 61 powdery mildew resistant RILs had *T. boeoticum* specific alleles, either at both the loci or at either of the two loci. Out of these 60 powdery mildew resistant RILs, 14 had *T. boeoticum* alleles for the flanking markers at locus *PmTb*7A.1, 17 PM resistant RILs had *T. boeoticum* allele for flanking markers at locus *PmTb*7A.2 and 29 RILs had *T. boeoticum* specific alleles at both the loci. All the susceptible RILs, except for two, had *T. monococcum* alleles for both the flanking markers at both the loci. The two susceptible RILs showed the *T. boeoticum* specific allele at the locus *PmTb7A*.2 but *T. monococcum* specific allele at the locus *PmTb7A*.1. *Bgt* isolates 07201 and 07302 are virulent on Axminster/8*CC or MIN which carry *Pm1* alleles *Pm1a* and *Pm1c*, respectively. These isolates showed avirulent reaction on the 17 RILs which had the Tb5088 allele at *PmTb*7A.2 locus (which corresponds to *Pm1*) indicating that Tb5088 carries a *Pm1* allele different from *Pm1a* and *Pm1c* and another gene which is novel.

Discussion

Powdery mildew had been a major wheat disease in hill regions of India but also appeared sporadically in the plains of India. However, in recent years its incidence has increased especially in the foothills of the northwestern plains of India. Breeding wheat varieties resistant to powdery mildew has not been the focus in breeding programmes so far, because of the difficulties for screening in segregating generations. Molecular markers, however, can be employed for breeding powdery mildew resistant varieties. In the present study, we identified a *T. boeoticum* accession that was resistant to powdery mildew isolates of Switzerland, France, and

USA and PM isolates present in plains as well as the Northern and Southern hill regions of India (our unpublished data). Identification of closely linked markers will help in transferring these resistance genes from *T. boeoticum* into tetraploid and hexaploid wheat cultivars. The *Tb*5088 accession showed an immune reaction to all the *Bgt* isolates tested. Inheritance studies, based on the evaluation of 148 RILs against three *Bgt* isolates, showed a nearly 3:1 segregation ratio indicating that *T. boeoticum* had two independently segregating genes for powdery mildew resistance. QTL mapping, using 153 markers on chromosome 7A showed that both genes are located on the long arm of chromosome 7A. *Pm1* is one of the several powdery mildew resistance genes so far mapped on chromosome 7AL of wheat. However, the resistant reaction of *Tb*5088 and RILs having Tb5088 allele at locus *PmTb*7A.2 against all the *Bgt* isolates indicated that the gene(s) identified in the *Tb*5088 are different from *Pm1a* and *Pm1c*.

Several PM resistance genes including *Pm1, Pm9, Pm37, mlRD30, Mlm2033, Mlm80 and NCA6* have been mapped on chromosome 7AL (Yao et al. 2007; Miranda et al. 2007). *Pm1* has been shown to have multiple alleles including *Pm1a, Pm1b, Pm1c, Pm1d and Pm1e* (McIntosh et al. 2008). *Pm1b* and *Pm1c* were transferred from *T. monococcum*, *Pm1d* from *T. spelta* and *Pm1c* and *Pm1e* from *T. aestivum*. *Mlm2033 and Mlm80*, identified in two different accessions of *T. monococcum* by Yao et al. (2007) have also been reported to be allelic to *Pm1*, based on the markers linked to these genes. *Pm37* has been reported to be closely linked to *Pm1*. The *Pm1* has been mapped in the terminal bin of 7AL linked to STS marker *MAG2185* derived from an RFLP marker *Xpsr680* and *MAG1759* derived from EST CD452874 (Yao et al. 2007). Markers *MAG2185, MAG1759* and STS marker derived from EST BE445506 (mapped in the terminal bin 7AL18-0.90-1.00), were found to be closely linked to *PmTb*7A.2 indicating that it might be allelic to *Pm1*. However, the second gene, *PmTb*7A.1 identified in the present study, mapping proximal to *PmTb*7A.2*,* is putatively a new gene, as none of the earlier catalogued *Pm* genes has been mapped at this location. *PmTb7A.1* and *PmTb7A.2* showed some deviation from independent assortment as was evident from the genotype of the flanking markers for both the loci. Out of a total of 61 PM resistant RILs, *T. boeoticum* specific allele was present at both the loci in 29 RILs, 14 RILs had markers flanking *PmTb7A.1* and 17 RILs had markers flanking *PmTb7A.2* against an expected ratio of 1:1:1. This deviation was expected as the two loci are less than 50cM apart. The location of the two powdery mildew resistance genes provide enough

scope for recovering recombinants with both the loci but with minimum of the wild species genome.

Resistance genes are very abundant in plant genomes, and they are not distributed randomly but rather appear to be clustered on particular chromosomes and many belong to tightly linked gene families (Hulbert et al. 2001). For example, a high proportion of actively transcribed wheat genes are clustered on chromosome arm 1AS (Gill et al. 1996), where resistance genes for *Pm3*, *Lr10* and *H9* are located. *Tm*14087 and *Tb*5088 also carry different but non-functional alleles of *Pm3* on 1A as was evident from limited sequencing studies (data not shown). Chromosome 2AL carries the powdery mildew resistance gene *Pm4* and a number of rust resistance genes *Yr1, Yr32 and Sr21* (McIntosh et al. 2008). Similarly, multiple genes for resistance to rusts in addition to powdery mildew are clustered on chromosome 7AL, including *Pm1*, *Pm9, Pm37, Lr20, Sr15, Sr22* and other temporarily designated genes (Ji et al. 2008). *Pm1* is tightly linked to the *Lr20-Sr15* gene complex (Neu et al. 2002). Presence of *Lr20* and/or *Sr15* could not be confirmed in the RIL population because both the parents were resistant to leaf rust and stem rust. However, a leaf rust resistance gene introgressed from *T. monococcum* acc. pau14087, into hexaploid wheat background has also been mapped on 7AL close to *Xcfa2019* (our unpublished results), to which *PmTb*7A.1 is also linked. A stem rust resistance gene *Sr22,* introgressed from *T. boeoticum*, has also been located in the same chromosomal region on 7AL linked to SSR marker *Xcfa2019* (Khan et al. 2005). Olson et al. (2010), however, identified another closer marker *Xwmc633* which was also observed to be closely linked to the left flanking marker of powdery mildew resistance gene *PmTb*7A.1 in the present study. Thus, the *PmTb*7A.1 might also be located in a gene rich region carrying powdery mildew, leaf rust and stem rust resistance genes.

We have generated a number of introgression lines in hexaploid wheat from crosses with *Tm*14087 and some RILs using *T. durum* as bridging species. These introgression lines had resistance to stripe and leaf rusts (Singh et al. 2007a; Chhuneja et al 2008). One of the RILs, designated as RIL101 has *T. boeoticum* specific alleles at both the loci *PmTb*7A.1 and *PmTb*-7A.2 but none of the introgression lines was resistant to powdery mildew when tested againt *Bgt* 07201 and *Bgt* 07302 isolates. Marker analysis of the introgression lines (data not given) showed that the chromosomal regions harbouring powdery mildew resistance genes in *T. boeoticum* were not transferred into hexaploid wheat.

The A^bA^b genome of *T. boeoticum*, recombines freely with the A genome of wheat and hence it is possible to transfer the target genes through backcrossing using *T. durum* as bridging species. For the transfer of target genes, *T. boeoticum* has to be crossed with a powdery mildew susceptible *T. durum* cultivar. The F_1 will be crossed with hexaploid wheat for the transfer of both powdery mildew resistance genes. Markers identified in the present study will be employed in the selection process. Limited backcrossing will be exercised initially to transfer powdery mildew resistance genes to desirable wheat background. Leaf rust and stripe rust resistance genes from *T. monococcum* and *T. boeoticum* have been transferred to bread wheat following the same strategy (Chhuneja et al. 2008; Singh et al. 2007a). Once powdery mildew resistance genes have been introgressed into a cultivated background these can be mobilized to other backgrounds through marker-assisted selection. *PmTb*7A.1, mapped in the present investigation is linked to an SSR marker *Xcfa2019* which can be easily used for marker-assisted selection. Similarly, *PmTb*7A.2 is linked to STS markers *MAG2185* and MAG1759 which are codominant markers and hence can prove useful for marker-assisted selection and gene pyramiding. Single, major resistance genes against wheat powdery mildew are not very durable because of virulence evolution of the pathogen. Therefore, the sustainable use of the two newly identified resistance genes will require combination of the two genes in the same genotype or combinations with other mildew resistance genes.

Positional cloning requires high resolution linkage map of the region carrying the target gene. Recently, a physical map of chromosome 3B was constructed using a Chinese Spring 3B-specific BAC library (Paux et al. 2008). Physical maps of other wheat chromosomes are being constructed. A high density map of the region on chromosome 7AL harbouring the two powdery mildew resistance genes could be developed, as and when the physical map and additional markers based on BAC end sequencing become available.

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Table 1. Screening for powdery mildew resistance with 14 *Bgt* isolates

Bgt isolate	*Tm*14087	*Tb*5088	RIL101	RIL130	PBW343	WL711	Axminster/8*CC (*Pm1a*)	M1N (*Pm1c*)
Bgt 95.9 Asosan	S	R	R	S	S	S	S	R
Bgt Ken 2-5	S	R	R	S	S	S	S	S
Bgt 98275	S	R	R	S	S	S	S	R
Bgt 97011	S	R	R	S	S	S	S	R
Bgt 98229	S	R	R	S	S	S	S	R
Bgt 07201	S	R	R	S	S	S	S	S
Bgt 07230	S	R	R	S	S	S	S	S
Bgt 07298	S	R	R	S	S	S	S	R
Bgt 07302	S	R	R	S	S	S	S	S
Bgt J2-1	S	R	R	S	S	S	IR	S
Bgt 07286	S	R	R	S	S	S	-	-
Bgt 85063	S	R	R	S	S	S	-	-
Bgt C3-1	S	R	R	S	S	S	-	-
Bgt 96244	S	R	R	S	S	S	S	R

R=resistant, S=susceptible, IR-intermediate resistance and -=data not available

Table 2. Segregation of *T. boeoticum/T. monococcum* RIL population for powdery mildew

Isolate	No. of RILs			χ^2 (3:1)[a]
	Total	Resistant[$]	Susceptible[$]	
Bgt 07201	143	97	46	3.91
Bgt 07302	142	97	45	3.39
Bgt 97011	143	97	46	3.91

[$]RILs with PM score of 0-5% were categorized as resistant and ones with >5% as susceptible.

Table 3. Mapping of a powdery mildew resistance gene in *T. boeoticum/T. monococcum* RIL population using linkage map of Singh et al. (2007b)

Bgt isolate	Chromosome	Marker interval	LRS	R^2 (%)
Bgt 07201	7AL	*Xcfa2019-Xgwm344*	32.6	28
Bgt 07302	7AL	*Xcfa2019-Xgwm344*	36.1	31
Bgt 97011	7AL	*Xcfa2019-Xgwm344*	31.1	27

Table 4. Mapping of powdery mildew resistance in *T. boeoticum/T. monococcum* RIL population based on an integrated map consisting of SSR, STS and DArT markers.

Bgt isolate	Locus	Marker interval	LRS[a]	R^2 (%)
Bgt 07201	*PmTb7A.1*	*wPt4553-Xcfa2019*	36.1	30
	PmTb7A.2	*MAG1759-MAG2185b*	38.2	32
Bgt 07302	*PmTb7A.1*	*wPt4553-Xcfa2019*	36.2	31
	PmTb7A.2	*MAG1759-MAG2185b*	40.6	34
Bgt 97011	*PmTb7A.1*	*wPt4553-Xcfa2019*	33.7	29
	PmTb7A.2	*MAG1759-MAG2185b*	31.3	27

[a]Threshold LRS for declaring a QTL highly significant ($P \leq 0.01$) were 23.1, 21.8 and 20.4 for powdery mildew infection data for isolates *Bgt* 07201, *Bgt* 07302 and *Bgt* 97011, respectively

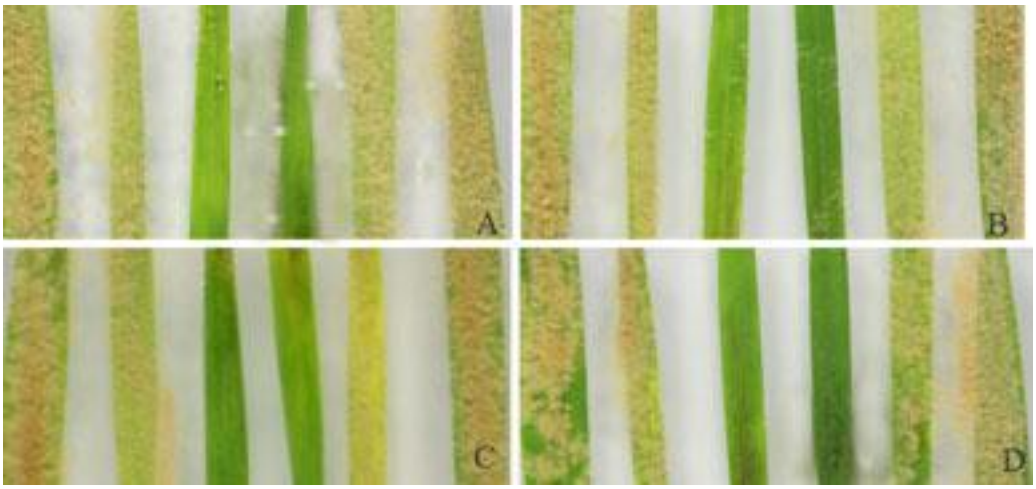

Fig. 1. Powdery mildew reaction to isolates *Bgt* 07201 (A), *Bgt* 07203 (B), *Bgt* Ken2-5 (C) and *Bgt* 98229 (D) using detached leaf method. Leaf segments 1-6 represent *T. aestivum* cv. WL711, *Tm*14087, *Tb*5088, RIL101, RIL130 and *T. aestivum* cv. PBW343

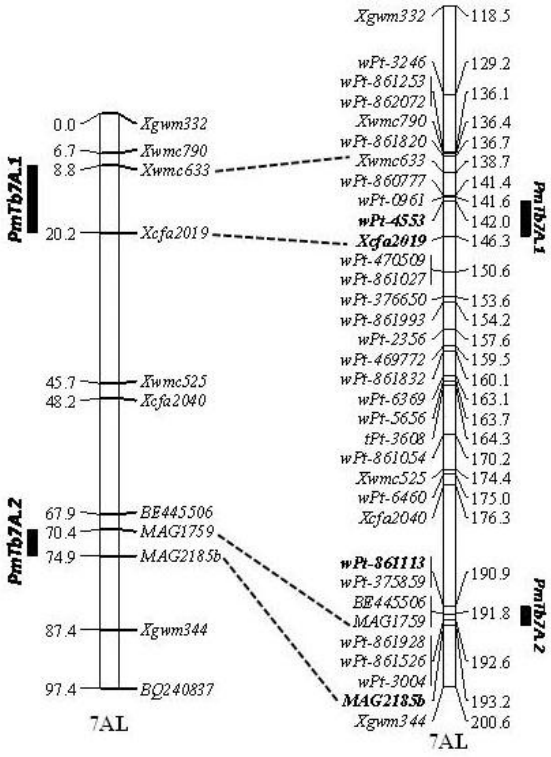

Fig. 2. Partial linkage maps of chromosome 7AL based on SSR, STS markers (**a**) and SSR, STS and DArt markers (**b**) showing chromosomal location of significant QTL for powdery mildew resistance in *T. boeoticum/T. monococcum* RIL population. Linkage maps were generated using linkage mapping software JoinMap®. Mapping for powdery mildew resistance was conducted using QTL mapping software MapManager QTXb20. Genetic distances in cM shown on right in **b** are the map positions for whole of the chromosome and on left in **a** are map positions of this linkage group only. Markers in bold indicate the intervals where the two powdery resistance genes map.